Aminophylline alters the core temperature response to acute hypoxemia in newborn and older guinea pigs

KIM C. CRISANTI AND JAMES E. FEWELL
Department of Physiology and Biophysics, The University of Calgary, Health Sciences Centre, Calgary, Alberta, Canada T2N 4N1

Crisanti, Kim C., and James E. Fewell. Aminophylline alters the core temperature response to acute hypoxemia in newborn and older guinea pigs. Am. J. Physiol. Regul. Integrative Comp. Physiol. 46: R829–R835, 1999.—In newborns and adults of a number of species, exposure to acute hypoxemia produces a “regulated” decrease in core temperature, the mechanism of which is unknown. The present experiments were carried out on chronically instrumented newborn (5–10 days of age; n = 27) and older (25–30 days of age; n = 23) guinea pigs to test the hypothesis that adenosine mediates this regulated decrease in core temperature. During an experiment, core temperature was measured by biotelemetry from animals studied in a thermocline during a control period of normoxemia, an experimental period of normoxemia or acute hypoxemia (fraction of inspired oxygen 0.10), and during a recovery period of normoxemia after an intraperitoneal injection of 10 mg/kg aminophylline (i.e., a nonspecific adenosine antagonist) or vehicle. Core temperature decreased significantly during hypoxemia after vehicle in both newborn and older guinea pigs. After aminophylline, however, newborn guinea pigs failed to significantly decrease their core temperature, whereas older guinea pigs exhibited an attenuated yet significant core temperature decrease during hypoxemia. Our data support the hypothesis that adenosine plays an age-dependent role in mediating the regulated decrease in core temperature that occurs in newborn and older guinea pigs during acute hypoxemia.

Methods

Fifty Hartley strain guinea pigs were studied. Each pup, born by spontaneous vaginal delivery, was housed with its mother and siblings in the vivarium of the University of Calgary's Animal Resource Center (22 ± 1°C, 20–30% relative humidity, and 12:12-h light-dark cycle). Although 22°C is below the reported thermoneutral zone of newborn guinea pigs (10), each pup had the opportunity to select its ambient temperature between experiments by huddling with its siblings and/or mother (i.e., behavioral thermoregulation).

Surgical preparation. Each guinea pig underwent one operation before study. Within 2–3 days of an experiment, each pup was anesthetized by inhalation of halothane (2.0% for induction and for maintenance) in oxygen. A paramedian laparotomy was done, and a battery-operated biotelemetry device (PhysioTel TA10ETA-F20; Data Sciences International, St. Paul, MN) was inserted in the peritoneal cavity for later measurement of core temperature. After surgery, the pups were returned to their mother for recovery.

All surgical and experimental procedures were carried out in accordance with the Guide to the Care and Use of Experimental Animals provided by the Canadian Council on Animal Care and with the approval of the Animal Care Committee of the University of Calgary.

Experimental protocol. For an experiment, each pup was removed from its mother and siblings, weighed, and placed in a thermocline into which flowed room air for a stabilization period of ~1 h. At the end of this stabilization period of normoxemia, measurements were made during a control period. A period of five consecutive measurements at 2-min intervals in which core temperature did not vary more than ±0.2°C was considered to be a suitable control period. After control measurements, the guinea pig was removed from the thermocline and given an intraperitoneal injection of aminophylline or vehicle. 

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phylline or an equal volume of vehicle. The animal was then returned to the thermocline and monitored for an additional 30 min. Each animal then underwent a 60-min experimental period of normoxemia or acute hypoxemia. After the experimental period, each animal underwent a 30-min recovery period of normoxemia. Dependent variables were recorded at 6-min intervals during the experimental and recovery periods.

Two age groups of animals were studied. A newborn group of 27 guinea pigs weighing 117 ± 19 g was studied between 5 and 10 days of age, and an older group of 23 guinea pigs weighing 278 ± 29 g was studied between 25 and 30 days of age. The animals in each age group were randomly assigned to one of the following four experimental groups: experimental group I received an intraperitoneal injection of vehicle after the control period and experienced normoxemia during the experimental period (n = 6 newborn; n = 5 older); experimental group II received an intraperitoneal injection of vehicle after the control period and experienced hypoxemia during the experimental period (n = 5 newborn; n = 5 older); experimental group III received an intraperitoneal injection of aminophylline after the control period and experienced normoxemia during the experimental period (n = 8 newborn; n = 7 older); and experimental group IV received an intraperitoneal injection of aminophylline after the control period and experienced hypoxemia during the experimental period (n = 8 newborn; n = 6 older).

Condition of observations. The thermocline used in our experiments consisted of a sealed perspex cylinder (2 m long, internal diameter 0.12 m) with a plastic grid along the bottom into which flowed room air. A linear temperature gradient from 10 to 40°C was produced by circulating hot and cold water (Endocal Refrigerated Circulating Bath RTE-8DD; Neslab, Newington, NH) in two copper coils wrapped around the cylinder. Gas of the desired oxygen concentration flowed through the thermocline at a constant rate (i.e., room air at 1.412 l/min; 10% oxygen in nitrogen at 1.490 l/min). Each time the gas mixture was changed, the thermocline was flushed by increasing the gas flow rate to ~8 l/min for 10 min. We have previously shown that decreasing the fraction of inspired oxygen from 0.21 to 0.10 produces hypcapnic hypoxemia in young guinea pigs, with the arterial oxygen and carbon dioxide pressures decreasing from 80 ± 9 to 24 ± 4 mmHg and 28 ± 3 to 5 ± 4 mmHg, respectively (6).

Experimental measurements and calculations. For measurement of core temperature, platform antennas (PhysioTel CTR 86; Data Sciences International) that received the output frequency (Hz) from the previously implanted biotelemetry device were placed under the thermocline. The received output was then fed into a peripheral processor (Dataquest III; Data Sciences International) connected to an IBM computer. Selected ambient temperature was determined by observing the position of the guinea pig in the thermocline. Oxygen consumption was calculated by the difference between the inflow and outflow (dry) oxygen concentration (Applied Electrochemistry S-3A/I O2 Analyzer; Ametek, Pittsburgh, PA) and the flow rate.

Aminophylline. Aminophylline anhydrous (USP; Sel-win Chemicals, Vancouver, BC) was used in a dose of 10 mg/kg body wt. Normal saline was used as vehicle. In preliminary
experiments, we found that intraperitoneal administration of 10 mg/kg body wt aminophylline produced plasma theophylline concentrations of 50 to 80 µmol/l in both newborn and older guinea pigs, which remained stable for a period from 30 to 90 min after injection (i.e., the duration of our experimental period of normoxemia or hypoxemia). This plasma concentration of theophylline, which is sufficient for adenosine receptor antagonism, inhibits <15% of cAMP phosphodiesterase activity (12, 34).

Statistical analysis. Statistical analysis was carried out using a four-factor ANOVA for repeated measures followed by a Student-Newman-Keul’s multiple comparison test to determine if age, drug, gas, or time affected core temperature, selected ambient temperature, or oxygen consumption. All results are reported as means ± SD, and P < 0.05 was considered to be of statistical significance.

RESULTS
With vehicle, core temperature decreased significantly during acute hypoxemia in both newborn and older guinea pigs (Fig. 1). After aminophylline, however, the newborn guinea pigs failed to significantly decrease their core temperature, and the older guinea pigs exhibited a significant yet attenuated decrease in core temperature during hypoxemia. Neither vehicle nor aminophylline had a significant effect on baseline core temperature during normoxemia in either age group of animals (Fig. 2).

SELECTED AMBIENT TEMPERATURE DURING ACUTE HYPOXEMIA IN GUINEA PIGS

DISCUSSION
Our data provide insight into possible mechanisms of thermoregulatory control in newborn and older guinea pigs during acute hypoxemia and during normoxemia. A novel finding in our study was that, although aminophylline did not alter basal core temperature in newborn and older guinea pigs during normoxemia, it did alter their core temperature response to acute hypoxemia. After aminophylline, newborn guinea pigs failed to decrease their core temperature, and older guinea pigs exhibited an attenuated yet significant decrease in

![Fig. 2. Core temperature before, during, and after an experimental period of normoxemia in newborn (A and B) and older (C and D) guinea pigs after an ip injection of vehicle (A and C) or aminophylline (B and D). N, experimental period (normoxemia).]
core temperature during hypoxemia. Thus our data provide evidence that adenosine does not exert a tonic influence on the central nervous system thermoregulatory "set point" under basal conditions in newborn and older guinea pigs. Furthermore, our data support the hypothesis that adenosine plays a role in mediating the regulated decrease in core temperature that occurs in newborn and older guinea pigs during exposure to acute hypoxemia.

Intraperitoneal administration of theophylline in the form of aminophylline, which is slightly more selective for the adenosine A1 receptor subtype than for the adenosine A2 receptor subtype (34) and readily crosses the blood-brain barrier (18), did not alter core temperature during normoxemia in either newborn or older guinea pigs. This is in agreement with the data of Kandasamy and Williams (16) who showed that intracebroventricular injection of either 10 or 30 µg of theophylline did not significantly alter core temperature in chronically instrumented, adult male guinea pigs. Furthermore, previous experiments carried out to determine whether or not adenosine influences basal thermoregulatory functions mediated by the preoptic anterior hypothalamus have shown that microinjection of adenosine antagonists into the preoptic anterior hypothalamus of rats (34) and rabbits (38) does not alter core temperature. Thus our data and the data of others provide evidence that adenosine does not exert a tonic influence on the central nervous system thermoregulatory set point under basal conditions.

Oxygen consumption increased after the intraperitoneal administration of aminophylline during normoxemia in newborn but not in older guinea pigs. Given that we did not observe general behavioral excitation in either the newborn or older guinea pigs as has been reported to occur in some species [e.g., rat (20, 33)], it is likely that the increase in oxygen consumption resulted from an age-dependent effect of theophylline on nonshivering thermogenesis in brown adipose tissue. Adenosine is known to attenuate (29, 31, 37) and methylxanthines are known to accentuate (9) catecholamine-stimulated nonshivering thermogenesis in brown fat cells studied in vitro. Furthermore, the long-acting adenosine analog N6-(2-phenylisopropyl)adenosine has been shown to inhibit nonshivering thermogenesis in brown fat cells studied in vitro. The age-dependent nature of the oxygen consumption response to theophylline is not surprising given the precipitous decline in uncoupling protein in brown adipose tissue and the replacement of nonshivering thermogenesis by shivering thermogenesis that occurs over the first 3 wk of postnatal life in guinea pigs (3, 25). It is likely that the transient decrease in selected ambient temperature that was observed in newborn guinea pigs after the intraperitoneal adminis-

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Fig. 3. Selected ambient temperature before, during, and after an experimental period of normoxemia in newborn (A and B) and older (C and D) guinea pigs after an ip injection of vehicle (A and C) or aminophylline (B and D). *P < 0.05 vs. C.
Fig. 4. Selected ambient temperature before, during, and after an experimental period of acute hypoxemia in newborn (A and B) and older (C and D) guinea pigs after an ip injection of vehicle (A and C) or aminophylline (B and D).

Fig. 5. Oxygen consumption before, during, and after an experimental period of normoxemia in newborn (A and B) and older (C and D) guinea pigs after an ip injection of vehicle (A and C) or aminophylline (B and D). *P < 0.05 vs. C.
tration of aminophylline prevented a forced increase in core temperature that has been reported to occur in some species [e.g., rat (20, 33)].

With vehicle, core temperature decreased significantly during acute hypoxemia in both newborn and older guinea pigs. After aminophylline, however, the newborn guinea pigs failed to significantly decrease their core temperature, and the older guinea pigs exhibited a significant yet attenuated decrease in core temperature during hypoxemia. Given that intraperitoneal administration of 10 mg/kg body wt aminophylline produced plasma theophylline concentrations of 50–80 µmol/l, which is sufficient for adenosine receptor antagonism (12, 34), our data allow us to suggest that additional factors are playing a role in mediating the core temperature response in the older guinea pigs. It is unlikely to be the endogenous opioids, as our previous experiments have shown that administration of naloxone, a nonspecific opioid receptor antagonist, does not significantly alter the core temperature response to acute hypoxemia in either newborn or older guinea pigs (7). Furthermore, neither afferents from the carotid baroreceptors/chemoreceptors (11, 13, 14, 21), an intact cerebral cortex (28), nor arginine vasopressin (5) appear to be important factors in mediating the core temperature response to acute hypoxemia.

After vehicle, oxygen consumption stayed the same or increased during hypoxemia in newborn and older guinea pigs that were studied at their thermoneutral temperature. This is in keeping with the early results of Hill (15) who carried out experiments on adult guinea pigs and found that oxygen consumption did not change during acute hypoxemia when the animals were studied at their thermoneutral temperature. Interestingly, Hill found that core temperature did not change when the adult guinea pigs were exposed to acute hypoxemia and studied at their thermoneutral temperature. This is different from our results and perhaps emphasizes the different strategies that newborn and older guinea pigs utilize to cope with a decreased oxygen level compared with that of an adult guinea pig.

In summary, our data support the hypothesis that adenosine plays a role in mediating the regulated decrease in core temperature that occurs in guinea pigs during acute hypoxemia. After aminophylline, newborn guinea pigs failed to significantly decrease their core temperature, whereas older guinea pigs exhibited an attenuated yet significant core temperature decrease during hypoxemia. The additional mechanisms responsible in part for mediating the core temperature response to acute hypoxemia in the older guinea pigs warrant further investigation.

Fig. 6. Oxygen consumption before, during, and after an experimental period of acute hypoxemia in newborn (A and B) and older guinea pigs (C and D) after an ip injection of vehicle (A and C) or aminophylline (B and D). *P < 0.05 vs. C.
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Address for reprint requests and other correspondence: J. E. Fewell, Heritage Medical Research Bldg. 206, The Univ. of Calgary, 3330 Hospital Dr. N.W., Calgary, Alberta, Canada T2N 4N1 (E-mail fewell@gacs.ucalgary.ca).

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